REMARKS

This Response is being made following a telephone interview with the Examiner, which occurred on 19 May 2005. The amendments above, and the following remarks are in accordance with the understandings regarding allowable subject matter, as detailed in said communication. Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-16, 19 and 21-62 are in this case. Claims 8-14, 21-44 and 53-60 were withdrawn under a restriction requirement as drawn to a non-elected invention. Claims 1-7, 15, 16, 19, 45-52, 61 and 62 have been rejected. Claims 3, 15, 16, 19, 45-52, 61 and 62 have now been cancelled without prejudice, rendering moot the Examiner's rejection thereof. Claims 1, 2, 4, and 5 have now been amended.

Priority

The Examiner has noted that the amendment filed on 26 November, 2004 regarding priority is deficient in pointing out the relationship between PCT/IL00/00514 and Application No. 09/385,411. An amended paragraph including the required information is provided herein, thereby overcoming the Examiner's objection.

35 U.S.C. § 112, 2nd Paragraph, Rejections

The Examiner has rejected claims 2 and 16 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to point out and distinctly claim the subject matter which applicants regard as the invention. The Examiner's rejections are respectfully traversed. Claim 16 has now been cancelled, rendering moot the Examiner's rejection thereof. Claim 2 has now been amended.

The Examiner has stated that the term "substantially lacks ribonucleic activity" in claims 2 and 16 renders the claim indefinite. Claim 2 has now been amended, and now recites: "...devoid of ribonucleolytic activity...". Support for such limitation can be found throughout the instant specification, for example:

> It will be appreciated that utilizing a T2-RNase, either directly or expressed from a polynucleotide, which displays a

> desired activity and yet is devoid of, or repressed in, ribonucleolytic activity is particularly advantageous since ribonucleolytic activity can produce undesired side effects in a subject. (page 21, lines 22-26)

In view of the abovementioned amendments, Applicant believes to have overcome the 35 USC 112, second paragraph rejections.

35 U.S.C. § 112, 1st Paragraph, Rejections

The Examiner has rejected claims 1-7, 15, 16, 19, 45-52 and 61-62 under 35 U.S.C. § 112, first paragraph, for lack of written description. The Examiners rejections are respectfully traversed. Claims 3, 15, 16, 19, 45-52, 61 and 62 have now been cancelled, rendering moot the Examiner's rejection thereof. Claims 1, 2, 4 and 5 have now been amended.

Referring now to Item 5 on page 3 of the communication of January 12, 2005, the Examiner states that the claims read on the use of T2 family proteins having an actin binding activity, that the genus of T2 family proteins is highly variant, that the specification discloses actin binding activity in *A. niger* T2 RNase alone, and that the specification fails to disclose nucleotide sequence information for *A. niger* T2 RNase. Further, the Examiner has stated that "It is unclear...whether any other ribonuclease of the T2 family has actin binding activity." and "there is no evidence of record that member[s] of the T2 ribonuclease family other than RNase B1 would also bind actin" (page 10 of the instant communication).

Regarding actin binding activity of T2 RNases, Applicant wishes to point out that, as discussed in the telephone interview, and as detailed on pages 18 (bottom paragraph) and 19 (top paragraph) of the communication of November 22, 2004, actin binding has been detected in T2 RNases of diverse origins. Regarding the new data provided in Professor Shoseyov's Declaration and the enclosed Appendix, Applicant submits that this data has been presented in the telephone interview, and it has been agreed that this new data supports the fact that all T2 Rnases have actin binding activity. Table I below demonstrates the actin binding activity of T2 RNases from the broadest range of phylogenetically diverse organisms, ranging from primitive bacteria, fungi which are primitive eukaryotes and human.

Table I- T2 RNase actin binding activity

Origin of RNase **Example**

Origin of RNase	**Actin Binding**	Pollen Tube Elongation Inhibition	HUVEC Angio-Genesis Inhibition	Cancer Cell Growth Inhibition	Tumor Growth Inhibition	In vivo Angiogenesis Inhibition
A. niger RNase B1	+	+	+	+	+	+
A. oryzea RNase T2	Not Available	+	+	Not Available	Not Available	Not Available
E. coli Rnase I	+	+	+	+	Not Available	Not Available
RNase 6PL (Human T2 Rnase)	+	+	+	+	+	Not Available

Not Available = experiment was not performed

Specific actin binding activity of *A. niger* RNase B1 has been demonstrated in pollen (Figs 10a and 10b of the instant specification), in solution (Fig. 11 of the instant specification), in HT-29 cancer cells (Figs. 28-30 of the instant specification), and on Western Blots (Fig 47 of US Provisional Application No. 60/613,702, filed 29 September 2004 and Fig. 1 of Appendix). Specific actin binding activity of *E. coli* RNase I has been demonstrated on Western Blots (Fig 47 of US Provisional Application No. 60/613,702, filed 29 September 2004 and Fig. 1 of Appendix). Specific actin binding activity of human RNase 6PL has been demonstrated in Western Blots (Fig 47 of US Provisional Application No. 60/613,702, filed 29 September 2004 and Fig. 1 of Appendix). Actin binding of *A. oryzae* RNase T2 has not been assessed.

Thus, Applicants have demonstrated actin binding activity in the utmost diverse selection of T2 RNases.

The Examiner has stated (page 10 of the Official action of January 12, 2005) that one of ordinary skill in the art would not know how to use the T2 RNases and methods of the present invention without knowing whether said ribonuclease has actin binding activity. Applicant wishes to re-emphasize, as noted in the communication of 26 November 2004, that actin binding assays, as detailed in the instant specification are well known in the art, and are well within the scope of one of ordinary skill in the art. Thus, determination of the criteria of actin binding does

not require undue experimentation. Let alone, as detailed above, the fact that phylogenetically most distinct T2 RNases have acting binding activity.

Referring now to Item 6 on page 5 of the official communication dated January 12, 2005, the Examiner has rejected claims 1-7, 15, 16, 19, 45-52, 61 and 62 for lack of enablement. The Examiner has stated that the claims encompass preventing, treating, inhibiting, or reversing proliferation, colonization, differentiation or development of abnormally proliferating cells, such as tumor cells, in a subject by using any ribonuclease of the T2 family having actin binding activity substantially lacking ribonucleolytic activity, via various routes of administration. However, the Examiner asserts that the specification teaches only the use of RNase B1 for the treatment and inhibition of tumor growth such as colon cancer and melanoma, that therapeutic amounts of the T2 RNase would vary, and thus it would require undue experimentation to practice the present invention over the full scope of the invention as claimed.

Further, the Examiner has stated, on page 9, that the data regarding the preventive effect of RNase B1 on the DMH-induced colon cancer formation cannot be extrapolated to prevention of various naturally occurring tumors or proliferation...colonization and angiogenesis of abnormally proliferating cells in a mammalian subject by any ribonuclease of the T2 family having actin binding activity. Applicant wishes to point out that, as detailed hereinbelow, the instant specification includes numerous examples of such preventive effects of T2 RNase of diverse origin.

Applicant wishes to point out that while reducing the invention to practice, it was uncovered that RNase B1 binds to membrane bound actin in cells, and substantially decreases proliferation of cancerous cells and reduces the size, number, and malignant potential of tumors in-vitro and in-vivo. As discussed in the interview of 19 May, these findings have been substantiated for T2 RNase of diverse phylogenetic origin, within a broad variety of in-vitro and in-vivo disease models, and employing a number of different modes of administration. Table II below summarizes the therapeutic potential of T2 RNases. Regarding the new data provided in Professor Shoseyov's Declaration and the enclosed Appendix, Applicant

submits that this data has been presented in the telephone interview, and it has been agreed that this new data supports the the scope of the claims.

Table II- Therapeutic effects of T2 RNases of diverse origins

A: *A. niger* RNase B1

Cancer Cells	Host	Mode of Induction	Mode of RNase Administration	Results	Example No.
Colon Cancer	Rat	DMH	Minipump weeks 1-9	↓ ACF	4
			Oral weeks 1-11	↓ Tumor no. ↓ Tumor size ↓ Malignancy ↓ Vascularity	4
			Minipump weeks 12-17	↓ ACF ↓ Tumor no. ↓ Tumor size ↓ Malignancy ↓ Vascularity ↓ Microvessel Density	4, 13
			Oral weeks 12-17	↓ ACF	4
B16F1 melanoma solid tumor	BDF1	Intraperitoneal injection (i.p.)	Intraperitoneal injection (i.p.)	↓ Tumor no.	8
B16F10 metastatic melanoma	Balb/c	Intravenous injection	Intravenous injection	↓ Metastases	8
A375SM melanoma solid tumor	Balb/c nude mice	Subcutaneous injection	Intraperitoneal injection	↓ Tumor size ↓ MMP-2	9
A375SM metastatic melanoma	Balb/c nude mice	Intravenous injection	Intraperitoneal injection	↓↓↓ Lung metastases	9
HT-29 colon cancer xenograft	CD1 nude mice	Subcutaneous injection	Intravenous injection	↓ Tumor weight; volume	13
HT-29 colon cancer xenograft	CD1 nude mice	Subcutaneous injection	Intraperitoneal injection	↓ Tumor weight (dose dependent) T2 detected in basal cells of tumor	13; Appendix Fig 6A, 6B

LS174T cancer cells	Balb/c nude mice	Subcutaneous injection	Intraperitoneal injection (after tumor development)	↓ Tumor growth ↓↓↓ Tumor growth synergy with Taxol	15
HUVEC tube formation	In-vitro			↓ Tube formation	7, 20 of US Provisional Application 60/613,702
GELFOAM implant vascularization	Balb/c mice	Intraperitoneal implantation of GELFOAM + angiogenin	Intraperitoneal injection	↓ neovascularization	10

B: *A. oryzae* RNase T2

Cancer Cells	Host	Mode of Induction	Mode of RNase Administration	Results	Example No.
HUVEC tube formation	In-vitro			↓ Tube formation	7, 20 of US Provisional Application 60/613,702 Appendix Fig. 4

C: *E. coli* RNase I

Cancer Cells	Host	Mode of Induction	Mode of RNase Administration	Results	Example No.
HT-29 Clonogenic assay	In-vitro			↓ Colony formation	Appendix Fig. 5
HUVEC tube formation	In-vitro			↓ Tube formation	7, 20 of US Provisional Application 60/613,702 Appendix Fig. 4

D: Human RNase 6PL

Cancer Cells	Host	Mode of Induction	Mode of RNase Administration	Results	Example No.
HT-29 Clonogenic assay	In-vitro			↓ Colony formation	Appendix Fig. 5
HUVEC tube formation	In-vitro			↓ Tube formation	7, 20 of US Provisional Application 60/613,702 Appendix Fig. 4

HT-29 colon cancer xenograft	CD1 nude mice In vivo	Subcutaneous injection	Intraperitoneal injection	↓ Tumor weight (dose dependent) T2 detected in basal cells of tumor	Appendix Fig 6A, 6B

Thus, while Table I demonstrates actin binding in bacterial, fungal and human T2 RNase, Table II clearly shows that in a variety of in-vivo and in-vitro models, representing clonogenic growth (HT-29 hepatic cancer cells), angiogenesis (HUVEC tube formation, GELFOAM implants and DMH-induced tumor vascularization), solid tumor formation (A375SM and B16F1 melanoma; DMH-induced colon cancer; HT-29 colon cancer xenograft and LS174-T), and metastatic spread of tumor (A375SM and B16F1 metastatic melanoma), T2 RNase having actin binding activity and of diverse phylogenetic origin was effective in preventing, treating and/or inhibiting colonization, growth, development and metastatic spread of tumor cells in a subject, via various routes of administration.

Yet further, the preventive effects of T2 RNase, and the dissociation between anti-tumorigenic effects and ribonucleolytic activity, as disclosed in the instant specification and in the additional references presented herein, have been recently corroborated independently by scientists investigating human RNase 6PL. Acquati et al (Int J Oncol. 2005 May;26(5):1159-68, enclosed herein) analyzed the human tumor suppressor gene product RNASET2 (identical to RNase 6PL), a secreted glycoprotein. RNASET2 was found to significantly decrease metastatic potential of a cancer cell line in vivo, and moreover, this activity was not affected by a double point mutation targeted to ribonuclease catalytic sites, thus providing yet further evidence of the preventive and therapeutic effects of T2 RNase disclosed in the instant specification, distinct from and not associated with ribonucleolytic activity.

Referring now to page 6 of the communication dated 12 January 2005, the Examiner has stated that the specification is not enabling for the treatment and/or prevention of various tumors other than melanoma and colon cancer as disclosed, and any disease or disorder other than tumor, such as Hodgkin's disease, arthritis, rheumatoid arthritis, diabetic retinopathy...in a subject by using T2 RNase. As discussed in the telephone interview, in order to further expedite prosecution in this

case, Applicants have elected to amend claim 1 to include the limitation of "...treating a solid tumor...", and claim 4 to recite solid tumors:

> Claim 1. (Currently amended) A method of treating a solid tumor in a mammalian subject, the method comprising administering to the subject a therapeutically effective amount of a ribonuclease of the T2 family having an actin binding activity, thereby treating said solid tumor in the subject.
>
> Claim 4. (Currently amended) The method of claim 1, wherein said solid tumor is associated with a proliferative disorder or disease selected from the group consisting of papilloma, blastoglioma, Kaposi's sarcoma, melanoma, lung cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, astrocytoma, head cancer, neck cancer, bladder cancer, breast cancer, colorectal cancer, thyroid cancer, pancreatic cancer, gastric cancer, hepatocellular carcinoma, lymphoma, Hodgkin's disease and Burkitt's disease.

Solid tumors are commonly defined as any abnormal mass of tissue that usually does not contain cysts or liquid areas. Solid tumors may be benign (not cancerous), or malignant (cancerous). Different types of solid tumors are named for the type of cells that form them: sarcomas, carcinomas, and lymphomas. Leukemias (cancers of the blood) generally do not form solid tumors. In this regard, Applicants wish to point out that Hodgkin's disease and Burkitt's disease are commonly defined as lymphomas, or tumors of the lymph nodes and glands, and as such are correctly included in the group of disorders associated with solid tumors.

Thus, now amended claim 1 and all claims dependent therefrom read on a method for treatment of solid tumors in a mammalian subject by RNase T2 having actin binding activity, as demonstrated in the instant specification, and further supported by experimental data (see Tables I and II hereinabove).

Referring now to page 12, The Examiner has stated that RNase having actin binding activity (i.e. angiogenin) can promote, rather than inhibit tumor growth or formation in vivo, and that this adds to the unpredictability of whether a T2 RNase having actin binding activity can prevent or treat tumor growth and/or metastasis in a subject. Applicant wishes to point out that the reference to angiogenin was made in order to illustrate that the combination of a ribonuclease and actin binding activity is

not sufficient to confer the anti-tumorigenic properties taught in the present invention, and rather that while many members of the T2 ribonuclease family are known, only those having actin binding activity would be suitable for use in the methods and compositions of the present invention. As detailed hereinabove, now amended claims 1 and 15 include the limitations "...a ribonuclease of the T2 family having an actin binding activity", thus one of ordinary skill in the art would readily distinguish between an RNAse of the T2 family and RNAse of other types (such as RNase A) having actin binding activity.

Regarding routes of administration, the Examiner has stated (page 14 of the communication dated January 12, 2005) that there are "barriers before a protein can reach it's target cells, for example, layers of dermal cells, blood vessel...membranes,...extracellular matrix...and the blood brain barrier", and that "...effective amounts of RNase at the target cells could vary, therefore, one skilled in the art...would require undue experimentation..." to determine the effective amounts and routes of administration to provide therapeutic effect over the full scope of the invention.

Applicant wishes to point out that Table II illustrates the variety of routes of administration (intravenous, intraperitoneal, continuous (osmotic pump) and oral) that the instant inventors have found effective for T2 RNase inhibition of tumor growth and metastasis in vivo, using T2 RNase having actin binding activity from diverse origins. Further routes of administration are disclosed in detail in the instant specification, for example, direct delivery to a hollow organ via catheter, needle, or via implantation of an osmotic minipump:

> "In addition, a cancer or tumor present in a body cavity, such as in the eye... can receive a physiologically appropriate composition...containing an effective amount of a T2-RNase via direct injection with a needle or via a catheter or other delivery tube placed into the cancer or tumor afflicted hollow organ." (page 28, lines 22-37 of the instant specification)

Such administration is commonly used in the delivery of drugs, including protein and peptide compositions, to the brain, in order to overcome permeability limitations imposed by the blood-brain barrier. Further, the formulation of the T2

RNase having actin-binding activity of the present invention within liposomes, another well-known method of drug delivery, is described in detail in the instant specification:

> "Liposome or micelle encapsulated T2-RNase or a polynucleotide encoding same may be administered topically, intraocularly, parenterally, intranasally, intratracheally, intrabronchially, intramuscularly, subcutaneously or by any other effective means at a dose efficacious to treat the abnormally proliferating cells of the target tissue."(page 29, lines 19-26)

Regarding dosage, it will be appreciated that different target cells and different methods of administration may require determination of a range efficacious doses, however, the means and criteria for such determination of suitable dosages are well known to one of ordinary skill in the art (see page 30, lines 14-31 of the instant specification). Indeed, an assay for determining effective therapeutic dosage of T2 RNase is disclosed in the instant specification (see page 25, lines 10-24).

Thus, it is the Applicants strong opinion that the results brought herein provide conclusive evidence of the significant therapeutic effects of the actin-binding ribonucleases of the T2 family of the present invention. Thus, one of ordinary skill in the art in possession of the teachings of the present invention, would be capable, with a reasonable expectation of success, of treating solid tumors at various locations over the body associated with the indicated diseases or disorders, using the T2 ribonucleases having actin-binding activity of the present invention, and the methods disclosed therein.

In view of the above arguments and amendments, Applicant believes to have overcome the 35 U.S.C. § 112, first paragraph, rejections.

In view of the above amendments and remarks it is respectfully submitted that claims 1, 2, 4, and 5-7 are now in condition for allowance. Prompt Notice of Allowance is respectfully and earnestly solicited.

Respectfully submitted,

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Date: June 30, 2005.

Encl: Three month's extension fee.
Declaration of Prof. Oded Shoseyov
Appendix
Reference: Acquati et al.